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Effects of abscisic acid on bud dormancy in Rosa and Syringa

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Effect of abscisic acid on bud dormancy in *Rosa* and *Syringa*

by

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INTRODUCTION

Of all the areas of plant growth and development that have been investigated, dormancy is one area which is not clearly understood. Although some breakthroughs in the area of bud dormancy have been accomplished, an understanding of some of the physiological factors controlling bud dormancy are still unsolved.

Since the late 1930's, two major hypotheses on the regulation of bud dormancy have appeared. The first of these hypotheses was introduced by Malan (43), who reported that apple and pear buds contained precursors of auxin during the rest period, but that there were no precursors of auxin in cherry, apricot, peach, and almond buds. He concluded from his work that auxin was not directly influencing the rest period in fruit trees. Contrary to the reports of Malan (43) and Zimmerman (68), Eggert (22) and Kassem (37) reported that bud dormancy in pear and apple buds was controlled by the presence of a supra-optimal auxin concentration rather than a lack of auxin. One may conclude from these early investigations that there was much contradiction and confusion whether auxins were truly regulating bud dormancy.

Late in the 1940's the second hypothesis on the regulation of bud dormancy was introduced. Hemberg (28, 29, 30) theorized that a growth inhibitory substance was responsible for inhibiting bud break and reported finding an inhibitor in dormant

potato tubers and in dormant ash seedlings. He also found the level of this inhibitor increased during dormancy and decreased at the time of bud break. Hendershott and Bailey (32), Hendershott and Walker (33), Blommaert (6), and Dennis and Edgerton (17) reported finding this inhibitor in dormant peach flower buds. Robinson et al. (55) also reported finding this acid inhibitor in Acer pseudoplatanus. From the investigations of Hemberg (28, 29, 30), Robinson et al. (55) and others (1b, 6, 7, 10, 16, 27, 31), it would seem that this inhibitor called abscisic acid does play a role in regulating bud dormancy.

The major objective of this work was to determine if an exogenous application of abscisic acid applied in the form of a spray at two stages of development or an immersion application during cold storage would delay bud break of Rosa 'Helen Traubel,' Rosa 'First Prize,' and Syringa 'Monge.' The effect of abscisic acid on shoot length, fresh weight, and dry weight was also determined.

The second objective was to determine by means of a lettuce seed bioassay if the biological activity of abscisic acid decreased during cold storage. It is hoped from this study that one may gain a better understanding of the effect of this natural occurring inhibitor upon bud dormancy.

LITERATURE REVIEW

Abscisic Acid and Its Role in Dormancy

The first investigation on the regulation of bud dormancy was reported by Malan (43) who found precursors of auxin in apple and pear buds during the rest period, but found no precursors of auxin in cherry, peach, apricot, and almond buds. Zimmerman (68) reported that auxin was present in sprouting buds of apple. Eggert (22) and Kassem (37) reported that in spur buds of apple and in pear buds that a super optimal concentration of auxin was controlling rest. Eggert (22) also observed an increase in auxin level as bud activity began in the spring. Avery et al. (2) found no auxin in the terminal buds of Malus and Aesculus during dormancy. Bennett and Skoog (5) also reported no diffusible auxin in resting lateral buds of cherries or pears. Blommaert (6) reported finding no auxin from extracts of dormant peach flower buds taken from October through March.

Hemberg (28, 29, 30) theorized in 1947 that a growth inhibiting substance played a major role in regulating bud dormancy. He reported that in dormant potato tubers and in dormant terminal buds of ash that this inhibitor increased during the dormant period and decreased at the beginning of bud break. From his investigations, he also observed that upon extracting the inhibitor from the peripheral of the tuber and terminal buds of the ash seedlings, he was able to inhibit

auxin induced curvature of Avena seedlings. He also reported that this inhibitor was acidic in nature and, upon running a chromatograph in an isopropanol, ammonia, and water solvent, he found that the Rf value was similar to β-inhibitor. From his studies, he also showed that there was a negative correlation between seasonal changes with regard to dormancy and the level of the growth inhibiting substance.

Hendershott and Bailey (32) reported isolating an acid inhibitor in dormant peach flower buds. They showed that extracts from buds of two varieties of peaches collected from December through March increased in inhibitory concentration from December through February and decreased at time of bud break. They also observed upon chromatographing the extracts from the leaves and buds that this inhibitor had an Rf value of .7-.9, corresponding to the Rf value of β-inhibitor which had been isolated earlier by Bennet et al. (4). Peach buds (6), terminal buds of longleaf pine (1a), French lilac buds (27), sycamore leaves (54), and leaves of Acer negundo (36) showed seasonal changes in an acid inhibitor which increased during dormancy and decreased at the time of bud break. Gabr and Gutteridge (24) have also isolated this inhibitor from strawberry leaves.

Hendershott and Walker (33) observed in peach buds that this inhibitor was confined to the bud scales and did not appear in the flower parts until sepal opening. At the time of bud break, they observed that there was a decrease in the

inhibitor in both the bud scales and the primordia. Further investigation showed that this inhibitor found in the bud scales was responsible for keeping the buds dormant. The concentration of the inhibitor in the bud scales remained constant as long as the buds remained dormant, but the concentration of the inhibitor in the flower primordia fluctuated.

Steward and Caplin (58) confirmed Hemberg's work by isolating an inhibitor from dormant potato tubers and silver maple buds. Lane and Bailey (40) also isolated this inhibitor from dormant buds of silver maple. Steward and Caplin (58) also observed that this inhibitor, which at this time was referred to as B-inhibitor, increased during the rest period and decreased when the buds started to swell.

Varga and Ferenczy (62) reported that the peripheral of the potato tuber contained an ether soluble inhibitor that decreased at the end of the rest period.

From about 1953-63, work by Eagles (20), Phillips and Wareing (54), and Wareing et al. (65) drew new light on how photoperiodism influenced the regulation of this acid inhibitor which Hemberg had isolated in 1947.

Nitsch (47) and later Eagles (20) and Wareing et al. (65) established that photoperiod had a definite effect on the level of the inhibitor found in buds and leaves. Wareing et al. (65) reported that short days caused an increase in the inhibitor while long days caused a decrease. Phillips and Wareing (54) found that when extracts from leaves of birch seedlings growing

under short days were applied to leaves growing under long days, this inhibitor would:

> ". . . cause arresting of growth, and cause the vegetative buds to change to resting buds by changing the developing leaf primordia into bud scales."

It was concluded from their results that this inhibitor, present during short days, was produced in the leaf and then transmitted to the stem apex where it exerted its effect on the bud.

Robinson et al. (55) and Thomas et al. (61) also observed the same results in Acer pseudoplatanus as did Wareing et al. (65). They (55) reported that this ether soluble inhibitor inhibited sections of wheat coleoptiles. Upon chromatographing the extracts of leaves of Acer pseudoplatanus, they found that its Rf value was .6-.8 which closely corresponded to the earlier findings of Hemberg (28, 29, 30) and Blommaert (6).

Kawase (38) showed that the activity of this inhibitor increased in the leaves of birch seedlings growing under short days, but as the number of short days increased above two weeks, a gradual decrease in activity was found. He also found this inhibitor in roots and stems. Kawase as well as others (1, 6, 35) noticed that as the activity of the inhibitor decreased, there was an increase in a growth promoting substance which was believed to be gibberellic acid. Kawase's studies (38) indicated that two short day inductions were necessary before this inhibitor could be isolated from plants previously growing under long days. The presence of this

inhibitor was determined by means of the oat coleoptile straight growth test. Kawase concluded, as have others (21, 31, 33, 35, 36), that this inhibitor was antagonistic to gibberellic acid.

El-Antably *et al.* (23) reported that they could induce dormancy of *Ribes nigrum* by spraying the leaves with this endogenous inhibitor at 5 and 25 ppm, but were unsuccessful in inducing dormancy in sycamore and birch. They concluded that the negative results with sycamore and birch were due to insufficient penetration of the inhibitor into the plants. They (23) also observed that if dormant twigs of willow were placed in a solution of this inhibitor at 1.0 and 2.0 ppm, they would remain dormant. Upon application of GA_3 to dormant twigs, they overcame the effect of this inhibitor and started to grow.

Work contributed by Little and Edict (42), with regard to bud dormancy, was in ring porous deciduous trees of *Acer pseudoplatanus*, *Betula alba*, and *Salix viminalis*. They found that an exogenous application of this inhibitor at 50, 250, and 500 ppm caused a delay in bud break and inhibits cambial activity.

Young and Cooper (67) have reported delaying bud break in Red Blush grapefruit with applications of 100, 500, and 1000 ppm of this inhibitor.

It was in 1963 that Wareing *et al.* (65) proposed the term "dormin" for this growth inhibiting substance (which had been previously called β-inhibitor), which acted as an endogenous dormancy inducer. At about this same time, Phillips and

Wareing (54) shifted their study from photoperiodism to the mechanism of translocation of "dormin."

Phillips and Wareing (54) reported that in growing shoots of Acer pseudoplatanus, the content of dormin within the buds increased from the top to the base of the shoot; whereas in resting shoots, the content of dormin within the buds increased from the base to the top. They also observed that when a plant was decapitated and defoliated, dormin decreased in the axillary buds. With this decrease of activity, they found an increase in auxin content.

Dörffling (18), in his study of Acer pseudoplatanus, observed the same results as Phillips and Wareing (54) in their study on translocation of dormin. Dörffling (18) showed that if a lanolin paste of dormin was placed on the decapitated portion of a stem of Pisum sativum, bud break was inhibited.

Dörffling and Böttger (19), in another investigation, observed the transport of dormin in explants, petioles, and internode segments of Coleus. In younger stems (the first or second internode), movement was mainly basipetal while in older segments, transport of dormin was either in an acropetal or basipetal direction. He also observed that the velocity of dormin in petiole segments was approximately 24-36 mm/hr. Milborrow (46) in his investigation found that basipetal movement in petioles was approximately three times that of acropetal transport.

Hoad (34), Bowen and Hoad (8) and Lenton et al. (41) have reported locating dormin in both xylem and phloem of Salix viminalis. They noticed that under short days there was a decrease in activity in both the phloem and xylem. Hoad (34) also observed that the concentration of dormin was in the order of 10 ug/100 ml of phloem sap and 1-5 ug/100 ml of xylem sap.

It was concluded from the work of Dörffling (18), Dörffling and Böttger (19), Hoad (34) and Lenton et al. (41) that dormin could be actively transported through the plant.

Carns and Liu (11) in 1961 isolated an abscission accelerating substance from cotton burs and named it Abscisin I. Steveninck (57) reported a similar substance which accelerated flower drop in yellow lupine. Osborne (49) reported isolating diffusates from senescening leaves that accelerated abscission.

Okuma et al. (50) reported isolating an abscission compound from cotton fruit, but reported that this acid substance was chemically different from Abscisin I. Okuma et al. (50) reported applying this inhibitor to petiole stumps of cotton seedlings which caused an accelerating of abscission. They named this compound Abscisin II with an empirical formula of $C_{15}H_{20}O_4$.

Cornforth et al. (13) confirmed the structure of Abscisin II by synthesis and used optical rotatory dispersion (ORD) for its identification.

It was at this point that Cornforth et al. (13, 14), by chromatography and ORD, found that the extract from sycamore leaves growing under short days was the same abscission accelerating substance found in cotton burs. It was from this study that they concluded that dormin, which was supposedly responsible for regulating bud dormancy, was also the same inhibitor accelerating abscission which they had named Abscisin II.

In 1966 at the Sixth International Conference on Plant Growth Substances, the name "abscisic acid" was given to this endogenous growth inhibitor which had been previously called dormin and Abscisin II (1a).

Chemistry and Biosynthesis of Abscisic Acid

One of the first investigations of the chemistry of abscisic acid was led by Okuma et al. (50) and Wareing et al. (64). They (50) reported that abscisic acid had an empirical formula of $C_{15}H_{20}O_4$ and was soluble in aqueous sodium bicarbonate, chloroform, acetone, methanol and ethanol. They also observed that natural occurring abscisic acid melted at 160° C and had an absorption maximum in methanol at 252 mu. In contrast to the natural occurring abscisic acid which is designated (+)-ABA or S-ABA, the synthetic ABA is a racemate of equal proportion of (+)-ABA and the synthetic (-)-ABA or RS-ABA (1a, 66). The (±)-ABA melts at 190° C, is optically inactive, and is less soluble than the (+)-ABA. It should also be stated that the (+)- and (-)- enantiomers are indistinguishable,

polarized light or by their reaction to other optically active substances (66).

Cornforth et al. (13) were the first to confirm the structure of abscisic acid using ORD and further "showed that abscisic acid was dextrorotatory and had a sinister configuration around the asymmetric carbon." The use of ORD, as a means of identification, allowed for a quantitative and qualitative means for estimating the presence of abscisic acid. ORD can detect abscisic acid at a concentration as low as .2 µg in 7 ml of organic solvent.

Another technique for identification of abscisic acid was reported by Milborrow (46) who also used ORD and ultra-violet absorption to develop a "racemate dilution method." This method allowed for the loss of abscisic acid in the steps between initial extraction and the final measurement of its optical rotatory dispersion.

Another technique for identification of abscisic acid has been the use of gas-liquid chromatography. This method was first reported by Davis et al. (15) and later confirmed by Gaskin and MacMillan (25). Another group led by Lenton et al. (41) has also reported developing a similar technique for identification of abscisic acid using gas-liquid chromatography (GLC).

Besides the use of ORD, UV, and GLC, plant bioassays have been used as a means for identification. The inhibition of ∝amylase (12, 31), inhibition of seed germination (12, 31),

inhibition of coleoptile curvature (31), inhibition of Lemna minor (53), and acceleration of radish leaf senescence (9, 51) are all techniques that have been used to determine the presence of abscisic acid.

Other chemical characteristics of abscisic acid are that it is a colorless crystalline solid and dissolves very sparingly in water. Wareing and Ryback (66) have reported that ABA loses water and rearranges into several neutral inactive compounds in the presence of strong acids. Milborrow (45) has reported that abscisic acid is light sensitive and, in the presence of ultraviolet light, it is transformed into trans-isomer configuration.

The "biosynthetic" mechanism of abscisic acid is one which is still open to speculation. Noodle and Robinson (48) and Wareing and Ryback (66) have reported that mevalonic acid may be a precursor of abscisic acid. They have observed that C^{14}-mevalonic acid can be incorporated into abscisic acid in ripening fruits of strawberry, tomato, and avocado. They also speculated that farnesyl pyrophosphate may also be an intermediate in abscisic acid synthesis. Mercer and Pughe (44) have reported that abscisic acid does not inhibit incorporation of 2-C^{14}-mevalonic acid into sterols in maize leaf tissues. They have also reported that abscisic acid inhibits synthesis of isoprenoid compounds located in the plastids.

Another theory on the biosynthesis of abscisic acid is thought to be through a precursor such as violaxanthin. Taylor

and Smith (60) have reported that violaxanthin may be the specific cartenoid which is converted to abscisic acid because

> "the pigment is the most commonly occurring xanthophyll in leaf tissues, has a similar structure to abscisic acid and modification of the ring by the breaking of the epoxy linkage could result in the production of hydroxyl group in the 6 position and a double bond in the ring - both being modifications in the abscisic acid molecule."

Although the means by which abscisic acid is produced is still unsolved, it would seem from the information that is present at this time, that other possible means could arise that are more convincing than the present theories.

Mode of Action of Abscisic Acid

The first speculation on the mode of action of abscisic acid in regulating bud dormancy was believed to be caused by a competitive interaction with gibberellic acid. Such a speculation was based primarily on the fact that gibberellic acid could negate the effect of abscisic acid in many biological tests. Chrispeel and Varner (12) reported that abscisic acid inhibits α-amylase production in barley endosperm, but that gibberellic acid could counteract the effect of abscisic acid and cause the resumption of α-amylase production. Boo (7) has observed in dormant potato tubers that gibberellic acid caused a reduction in abscisic acid and resulted in bud break. Brian et al. (9) reported that gibberellic acid can counteract the effect of abscisic acid in accelerating senescence in leaf discs of radishes. El-Antably et al. (23) reported that

dormant buds previously treated with abscisic acid at 1.0 and 2.0 ppm were almost completely inhibited while partially overcome when gibberellic acid was applied at 10 ppm. Robinson et al. (55) also reported gibberellic acid counteracts the effect of abscisic acid in dormant potato tubers. Beevers (3) has reported that kinetin can counteract the effect of abscisic acid in leaf discs of nasturtium.

Kawase (38) reported that birch seedlings growing under short days followed by an application of gibberellic acid resulted in bud break. Numerous other reports (20, 21, 42) support the theory that abscisic acid competitively interacts with gibberellic acid.

Although much evidence supporting this theory has been presented, the work of Overbeek et al. (52) and Good (26) seems to show that abscisic acid and gibberellic acid are not competitively interacting. Good (26) reported that gibberellic acid cannot negate the effect of abscisic acid in dwarf corn shoots. He also showed that in lettuce seed germination tests, gibberellic acid was not effective in overcoming the inhibition of abscisic acid, whereas kinetin was effective.

Overbeek et al. (52) and Steward (59) have reported that only kinetin was able to counteract the effect of abscisic acid in growth of Lemna minor while gibberellic acid was ineffective.

It would seem from the results of Overbeek et al. (52) and Good (26) that there is little evidence to support the view

that abscisic acid is competitively interacting with gibberellic acid.

Another theory, supported by Wareing et al. (64), believes that the mode of action of abscisic acid in regulating bud dormancy is by affecting gibberellic acid biosynthesis. They have reported that when spinach plants were placed under one long day cycle, there was an increase in gibberellic acid; but, upon applying exogenous application of abscisic acid during a long day cycle, the gibberellic acid level remained the same as under short days. They have also found that when applying P^{32} to the leaf discs of Taxaxcum officinalis and Raphanus sativus which had been previously treated with abscisic acid resulted in the reduced incorporation of P^{32} into the soluble ribosome and heavy ribosome fraction. From these two studies they speculated that the primary effect of abscisic acid is "to inhibit RNA synthesis, this in turn results in the inhibition of enzyme synthesis and inhibition of biosynthesis of hormones."

Villiers (63) reported that in dormant seeds of Fraxinus, abscisic acid inhibits the incorporation of H^3-uridine and H^3-thymidine, but not leucine; while gibberellic acid stimulates H^3-uridine and thymidine.

Villiers (63) speculates that abscisic acid maintains dormancy by inhibiting the production of specific types of messenger-RNA which in turn inhibit the formation of specific proteins.

Staden and Bornman (56) also reported that abscisic acid affects nucleic acid metabolism in Spirodela.

A third view led by Overbeek et al. (52) speculates that abscisic acid maintains dormancy by inhibition of DNA synthesis. From their investigation, they have shown that abscisic acid inhibits growth of Lemna minor while benzyladenine stimulates its growth. They have also observed that abscisic acid inhibited nucleic acid metabolism in Lemna minor while benzyladenine stimulated it. Inhibition of the nucleic acid is initially on the DNA synthesis and not on the RNA, according to them.

Although there are major differences with regard to the role of abscisic acid in maintaining dormancy, it would seem that abscisic acid does affect some portion of the nucleic acid metabolism in its regulation of dormancy.

METHODS AND MATERIALS

Effect of Abscisic Acid as a Spray Application on *Rosa* and *Syringa*

Experiment 1

Syringa 'Monge' and *Rosa* 'Helen Traubel' were removed from cold storage on February 6, and all dead terminal and lateral buds removed. All plants were pruned of their larger roots and then planted in two gallon plastic pots in a soil mixture containing equal parts of soil, sand, and peat. Each plot in the experiment contained one 1-stem plant and two 2-stem plants.

Abscisic acid in the form of a crystal was placed in a 100-ml solution of water. A sonic vibrator was used for about 15-30 minutes, and abscisic acid was then converted to a potassium salt by the addition of 1N K_2CO_3 to a pH of 7. A surfactant, Triton 100 at a rate of 0.5%, was used as a wetting agent. The abscisic acid was then placed in two Hudson hand sprayers and stored at 35° F.

The plants were placed in the greenhouse where they received one of three treatments consisting of either a "dormant" spray, "active" spray, or a dormant-active combination. The concentrations used were 0, 100, and 200 ppm of abscisic acid.

In the case of the "dormant" application in lilacs, abscisic acid was applied on both the second and third days after the plants were placed in the greenhouse. The "active" spray was applied on both the sixth and seventh days after the

plants were placed in the greenhouse when the plants were showing signs of bud swell.

Roses received a "dormant" spray of abscisic acid on both the sixth and seventh days after being placed in the greenhouse. The "active" spray was applied on both the tenth and eleventh days after being placed in the greenhouse.

All spray applications were made early in the day when temperatures were about 70° F. Day temperatures ranged from 60-80° F, while night temperatures ranged from 60-65° F. All plants in the experiment received approximately 11 hours of natural light, followed by 5 hours of 100 foot candles of incandescent light.

Measurement of bud break of the terminal buds of Syringa 'Monge' was based on 50% of the total live terminal buds breaking on a single or multi-stem plant. Lateral bud break was based on 50% of the first six buds (single stem) or 12 buds (multi-stem) breaking. In cases where a sufficient number of buds did not break on the terminal or laterals, the plants were considered to be dormant and were not included in the data for shoot growth, fresh weight, or dry weight.

Measurement of bud break of Rosa 'Helen Traubel' was based on 50% of the buds breaking. In cases where two buds arose from one node, only one bud was counted. Malformation of buds and stems was noted.

At the end of the experimental period (4 weeks), plants were harvested. In the case of Syringa 'Monge' the shoot

growth of the terminals and laterals was measured. Shoots were then put in individual plastic bags and placed in a styrofoam cooler containing dry ice.

The plants were then removed from the plastic bag and the fresh weight was taken of the new terminal and lateral shoots. The plants were then placed on aluminum foil and placed in a dryer for 24 hours at 58° C. The lilacs were removed from the dryer where their dry weight was measured.

The procedure of harvesting and measuring was the same for roses.

Effect of Abscisic Acid as an Immersion Application on Rosa and Syringa

Experiment 2

Rosa 'Helen Traubel' and Syringa 'Monge' were removed from storage on March 14 and March 28 respectively. All dead and broken stems and roots were pruned. Any terminal or lateral buds which appeared dead were also removed.

In the case of roses, all plots consisted of one 1-stem plant and three 2-stem plants. Abscisic acid was prepared as stated for Experiment 1.

After all pruning was completed, Rosa 'Helen Traubel' and Syringa 'Monge' were then placed in 24"x48"x16" brown polyethylene bags in which moss sphagnum had been placed. Within each treatment, three lilacs were placed in each plastic bag while four roses were placed in each plastic bag. The plants were then assigned treatment numbers before being placed back

into cold storage. The treatments consisted of a 5-minute immersion application(s) of abscisic acid 1) 4 weeks before removal from storage, 2) 3 and 2 weeks before removal from storage, 3) 4 and 0 weeks before removal from storage, and 4) 0 weeks before removal from storage. An application of abscisic acid at 0 weeks before removal from storage refers to an application of abscisic acid at the time plants were removed from cold storage. The concentrations of abscisic acid used were 0, 200, and 400 ppm. The temperature ranged from 33-35° F for roses and 34-36° F for lilacs while in storage.

Prior to the application of abscisic acid in the form of a 5-minute immersion, both species were removed from cold storage two hours before treatment. Plants were allowed to dry for 30 minutes before being placed back into cold storage.

Upon removal from storage, the plants were examined for injury that might have been caused by abscisic acid during storage.

The roses and lilacs were then planted in two gallon plastic pots in an equal mixture of soil, sand, and peat, and placed in the greenhouse where they received approximately 11 hours of natural light, followed by 5 hours of 100 foot candles of incandescent light. Day temperatures ranged from 65-100° F while night temperatures ranged from 65-80° F.

Measurement of terminal bud break of lilacs was based on 50% of the terminal buds breaking on either a single or multi-stem plant. Lateral bud break was based on 50% of the first

four lateral buds breaking in a single stem plant and eight buds on a multi-stem plant. Measurement of bud break for Rosa was the same as in Experiment 1.

After four weeks in the greenhouse, plants were harvested and measured for new shoot length, fresh weight, and dry weight of new shoots. The same procedure in measuring for shoot length, fresh weight, and dry weight was taken, as had been previously done in Experiment 1 for both lilacs and roses.

Effect of Abscisic Acid as an Immersion Application on Bud Break of Rosa 'First Prize'

Experiment 3

Plants of Rosa 'First Prize' were removed from cold storage on May 2. All plants were pruned and potted as has been previously stated for Experiment 1. Plots consisted of two 2-stem plants.

Treatments in this experiment consisted of a 5-second, 1, 3, 6, and 9-minute immersion application in 400 ppm of abscisic acid. Upon completion of the treatments, the plants were placed in the greenhouse under the same environmental conditions as has been stated for Experiment 2.

At the end of the experimental period (4 weeks), bud break was determined. The basis for determining bud break was the same as in earlier experiments.

Effect of Abscisic Acid on *Lactuca sativa* 'Grand Rapids'

Experiment 4

Lettuce seed, *Lactuca sativa* 'Grand Rapids,' was used in this experiment to determine if there was a decrease in activity of abscisic acid in solution during cold storage.

Two identical solutions of abscisic acid were prepared as has been previously stated for Experiment 1. One of the solutions had been used previously for Experiment 2 while the other had not been used on any plant material. Both solutions were kept in the dark and under refrigeration at 36-38° F.

From these two solutions, identical concentrations consisting of 0, .5, 1.0, 2.5, 5.0, and 10.0 ppm were made.

Lettuce seeds were placed in a 250-ml glass beaker, containing 5% sodium hypochlorite, for 5 minutes and then rinsed twice with distilled water. The seeds were then placed on paper towels for 10 minutes to dry. Ten seeds were then placed in each 7-inch petri dish which contained a layer of No. 7 filter paper. Each treatment consisted of five replications. From each individual solution, 5 ml of abscisic acid were added to the petri dishes. The petri dishes were then placed in the dark for 72 hours where the temperature was 65° F. At the end of 72 hours, percentage germination and the length of the radicles were determined.

Germination was based upon the emergence of the radicle through the seed coat. The length of the radicle was measured

in centimeters. This test was repeated two weeks after the stock solution was initially prepared and again ten weeks later.

Statistical Analysis

The mathematical model for the statistical analysis of Experiments 1 through 3 is:

$$Y_{ij} = \mu + A_i + B_j + AB_{ij} + \epsilon_{ijk}$$

μ = mean of the population

A_i = effect of the ith treatment

B_j = effect of the jth block

AB_{ij} = effect of the ijth combination

$\epsilon_{ijk} \sim$ NID $(0, \sigma^2)$.

Experiment 1 consisted of a randomized complete block design which consisted of three concentration levels and three different times of application. Experiment 2 consisted of the same statistical design as Experiment 1 except that it consisted of four different times of application. Experiment 1 consisted of 12 plants/treatment with 3 plants in each of 4 blocks while Experiment 2 consisted of 12 plants/treatment with 3 plants in each of 4 blocks, in the case of lilacs. In the case of roses, it consisted of 16 plants/treatment with 4 plants in each of 4 blocks.

Experiment 3 consisted of a randomized complete block design which was composed of one concentration level and five

different time intervals. In all cases, 8 plants/treatment with 2 plants in each of 4 blocks were used.

Standard analyses of variance were followed by the "F" test which was used to determine statistical significance of concentration and time of application. The "F" value was calculated by dividing the error mean square by the mean square.

The least significant difference (L.S.D.) was calculated in all cases where the "F" value was significant. The formula for computing L.S.D. was:

$$\sqrt{\frac{2\,(\text{error mean square})}{\text{Number of observations}}} \left(t_{.05} \text{ at the given degree of freedom} \right)$$

RESULTS

Effect of Abscisic Acid as a Spray Application on Rosa

Effect of abscisic acid on bud break

'Helen Traubel' roses were sprayed with abscisic acid at two different growth stages to determine its effect on bud break, shoot elongation, and fresh and dry weight of new shoots.

Table 1 shows the effect of concentration of abscisic acid and time of application on bud break of Rosa 'Helen Traubel.' Abscisic acid was shown to inhibit bud break at the 1% probability level. The data indicate that time of application was not significant. The data also show a linear response in inhibition of bud break except in the case of the 6th and 7th day spray application of abscisic acid at 200 ppm. A response may not have occurred at this level because of a lack of penetration of abscisic acid into the buds and stems or possibly due to the presence of gibberellic acid counteracting the effect of abscisic acid. The presence of gibberellic acid counteracting the effect of abscisic acid at the time of bud break has been shown by Kawase (38), Blommaert (6) and Little and Edict (42). The 6th and 7th day spray applications of abscisic acid at 200 ppm resulted in a time of application x concentration interaction. Table 1 also shows that the 6th and 7th and 10th and 11th day spray applications of abscisic acid at 200 ppm were the most effective treatment in inhibiting bud break.

Table 1. Effect of abscisic acid on days to bud break of Rosa 'Helen Traubel' through 28 days

Concentration of ABA (ppm)	Two spray applications, days after removal from storage[a] (days)			
	6th & 7th[b]	10th & 11th[c]	6th & 7th[d] 10th & 11th	Means
Control	17.2	15.0	15.3	15.9
100	20.3	17.3	17.6	18.6[e]
200	16.5	20.2	23.4	20.0[f]
Means	18.0	17.5	18.8	18.1

Analysis of variance of bud break

Source of variation	D.F.	S.S.	M.S.	F
Replication	3	3.301	1.10	.1159
Concentration	2	105.72	52.86	5.56**
Time of application	2	10.09	5.04	.531
Time of application x concentration	4	119.92	29.98	3.15*
Error	24	227.83	9.49	
Total	35	466.88		

**Significant at .01 level.

*Significant at .05 level.

[a]Each treatment value is the mean of 12 plants.

[b]Applications of ABA made during dormant stage.

[c]Applications of ABA made at time of bud swell stage.

[d]Applications of ABA made at dormant and bud swell stage.

[e]LSD (.05) = 2.68.

[f]LSD (.01) = 3.63.

Effect of abscisic acid on shoot growth

Data in Table 2 show that abscisic acid did not inhibit shoot growth. Both time of application and concentration were not statistically significant. Although the concentrations were not significant, it did show that as concentration increased there was a slight decrease in shoot growth.

Effect of abscisic acid on fresh and dry weight

Fresh weight and dry weight of shoot growth are shown in Tables 3 and 4. The data indicate that concentration nor time of application had a significant effect on either fresh weight or dry weight. Although not significant, there was a slight decrease in dry weight as one increased the concentration of abscisic acid.

It can be concluded from this experiment that abscisic acid delayed bud break in Rosa but did not affect subsequent growth of the plant.

Effect of Abscisic Acid as a Spray Application on Syringa

Effect of abscisic acid on terminal and lateral bud break

The effect of abscisic acid on terminal and lateral bud break of Syringa 'Monge' in Tables 5 and 6 shows that time of application nor concentration inhibited bud break. Although not significant, abscisic acid at 100 and 200 ppm delayed terminal and lateral bud break. Greatest inhibition occurred at 200 ppm, while the 2nd and 3rd day spray applications

Table 2. Effect of abscisic acid on length of new shoot growth of Rosa 'Helen Traubel' through 28 days

Concentration of ABA (ppm)	Two spray applications, days after removal from storage[a] (cm)			
	6th & 7th	10th & 11th	6th & 7th 10th & 11th	Means
Control	15.1	16.1	15.9	15.7
100	10.1	16.6	14.5	13.7
200	14.9	11.6	13.2	13.2
Means	13.4	14.7	14.5	14.2

Analysis of variance of shoot growth

Source of variation	D.F.	S.S.	M.S.	F[b]
Replication	3	79.28	26.42	1.020
Concentration	2	40.51	20.25	.787
Time of application	2	12.95	6.47	.251
Time of application x concentration	4	99.81	24.95	.969
Error	24	617.70	25.73	
Total	35	850.27		

[a]Each treatment value is the mean of 12 plants.

[b]F value required at .05 = 3.01.

Table 3. Effect of abscisic acid on fresh weight of new shoot growth of Rosa 'Helen Traubel' through 28 days

Concentration of ABA (ppm)	Two spray applications, days after removal from storage[a] (gm)			
	6th & 7th	10th & 11th	6th & 7th 10th & 11th	Means
Control	5.0	4.0	4.4	4.47
100	2.6	4.3	3.8	3.58
200	4.5	4.7	4.1	4.47
Means	4.0	4.3	4.0	4.17

Analysis of variance of fresh weight

Source of variation	D.F.	S.S.	M.S.	F[b]
Replication	3	19.91	6.63	2.00
Concentration	2	6.21	3.10	.94
Time of application	2	.68	.34	.10
Time of application x concentration	4	7.88	1.97	.59
Error	24	79.09	3.29	
Total	35	113.80		

[a] Each treatment value is the mean of 12 plants.

[b] F value required at .05 = 3.01.

Table 4. Effect of abscisic acid on dry weight of new shoot growth of Rosa 'Helen Traubel' through 28 days

Concentration of ABA (ppm)	Two spray applications, days after removal from storage[a] (gm)			
	6th & 7th	10th & 11th	6th & 7th 10th & 11th	Means
Control	.79	.67	.67	.711
100	.49	.81	.59	.634
200	.56	.50	.62	.559
Means	.61	.66	.62	.630

Analysis of variance of shoot growth

Source of variation	D.F.	S.S.	M.S.	F[b]
Replication	3	.219	.073	.606
Concentration	2	.138	.069	.575
Time of application	2	.015	.007	.065
Time of application x concentration	4	.272	.068	.564
Error	24	2.89	.120	
Total	35	3.53		

[a]Each treatment value is the mean of 12 plants.

[b]F value required at .05 = 3.01.

Table 5. Effect of abscisic acid on days to terminal bud break of Syringa 'Monge' through 28 days

Concentration of ABA (ppm)	Two spray applications, days after removal from storage[a] (days)			
	2nd & 3rd	6th & 7th	2nd & 3rd 6th & 7th	Means
Control	7.2	7.9	7.3	7.5
100	7.2	8.1	7.7	7.7
200	10.1	7.6	8.8	8.8
Means	8.1	7.9	8.0	8.0

Analysis of variance on terminal bud break

Source of variation	D.F.	S.S.	M.S.	F[b]
Replication	3	3.33	1.11	.39
Concentration	2	12.72	6.36	2.28
Time of application	2	.38	.19	.06
Time of application x concentration	4	13.60	3.40	1.21
Error	24	66.96	2.79	
Total	35	97.00		

[a] Each treatment value is the mean of 12 plants.

[b] F value required at .05 = 3.01.

Table 6. Effect of abscisic acid on days to lateral bud break of Syringa 'Monge' through 28 days

Concentration of ABA (ppm)	Two spray applications, days after removal from storage[a] (days)			
	2nd & 3rd	6th & 7th	2nd & 3rd 6th & 7th	Means
Control	18.7	19.8	16.0	18.2
100	16.7	21.2	18.1	18.7
200	21.2	19.0	18.7	19.6
Means	18.9	20.0	17.6	18.8

Analysis of variance of lateral bud break

Source of variation	D.F.	S.S.	M.S.	F[b]
Replication	3	32.13	10.71	1.26
Concentration	2	13.31	6.65	.78
Time of application	2	35.57	17.78	2.10
Time of application x concentration	4	54.69	13.67	1.61
Error	24	202.59	8.44	
Total	35	338.31		

[a]Each treatment value is the mean of 12 plants.

[b]F value required at .05 = 3.01.

appeared to be the best time of application in inhibiting terminal bud break.

Effect of abscisic acid on terminal and lateral shoot growth

Data on terminal and lateral shoot growth appear in Tables 7 and 8. Neither time of application nor concentration is significant. Table 7 also shows that a concentration of abscisic acid at 100 ppm was more effective in stimulating shoot growth than the control.

Data on lateral shoot growth show an increase in shoot growth as the concentration of abscisic acid increased. With reference to the number of applications, an increase in inhibition of shoot growth occurred as the number of applications was increased. It can be concluded that four applications of abscisic acid were more effective than two applications in inhibiting lateral shoot growth.

Effect of abscisic acid on fresh and dry weight of terminal shoot growth

The effect of abscisic acid on fresh weight and dry weight appears in Tables 9 and 10. Neither time of application nor concentration was significant. A concentration of abscisic acid at 100 ppm at all spray application periods caused a slight increase in fresh weight, while at 200 ppm it caused a slight decrease in fresh weight as compared to the control.

With reference to dry weight, a concentration of abscisic acid at 100 ppm at all spray periods caused a slight increase

Table 7. Effect of abscisic acid on length of new terminal shoot growth of Syringa 'Monge' through 28 days

Concentration of ABA (ppm)	Two spray applications, days after removal from storage[a] (cm)			
	2nd & 3rd	6th & 7th	2nd & 3rd 6th & 7th	Means
Control	7.3	7.4	7.9	7.6
100	8.8	8.9	8.9	8.9
200	6.2	8.4	7.2	7.3
Means	7.5	8.2	8.0	7.9

Analysis of variance of terminal shoot growth

Source of variation	D.F.	S.S.	M.S.	F[b]
Replication	3	8.61	2.87	.606
Concentration	2	17.39	8.69	1.830
Time of application	2	3.54	1.77	.374
Time of application x concentration	4	6.70	1.67	.354
Error	24	113.62	4.73	
Total	35	149.90		

[a]Each treatment value is the mean of 12 plants.

[b]F value required at .05 = 3.01.

Table 8. Effect of abscisic acid on length of new lateral shoot growth of Syringa 'Monge' through 28 days

Concentration of ABA (ppm)	Two spray applications, days after removal from storage[a] (cm)			
	2nd & 3rd	6th & 7th	2nd & 3rd 6th & 7th	Means
Control	5.1	5.7	5.3	5.4
100	5.3	7.4	5.6	6.1
200	6.5	7.0	5.5	6.3
Means	5.6	6.7	5.5	5.9

Analysis of variance of lateral shoot growth

Source of variation	D.F.	S.S.	M.S.	F[b]
Replication	3	25.98	8.66	2.50
Concentration	2	5.77	2.88	.83
Time of application	2	10.90	5.45	1.57
Time of application x concentration	4	5.35	1.33	.38
Error	24	82.89	3.45	
Total	35	130.92		

[a]Each treatment value is the mean of 12 plants.

[b]F value required at .05 = 3.01.

Table 9. Effect of abscisic acid on fresh weight of new terminal shoot growth of Syringa 'Monge' through 28 days

Concentration of ABA (ppm)	Two spray applications, days after removal from storage[a] (gm)			
	2nd & 3rd	6th & 7th	2nd & 3rd 6th & 7th	Means
Control	1.3	1.6	1.5	1.5
100	1.7	1.8	2.1	1.9
200	1.2	1.7	1.4	1.4
Means	1.4	1.7	1.7	1.6

Analysis of variance of fresh weight

Source of variation	D.F.	S.S.	M.S.	F[b]
Replication	3	.374	.124	.374
Concentration	2	1.480	.743	2.233
Time of application	2	.631	.315	.949
Time of application x concentration	4	.393	.098	.295
Error	24	7.98	.332	
Total	35	10.86		

[a] Each treatment value is the mean of 12 plants.

[b] F value required at .05 = 3.01.

Table 10. Effect of abscisic acid on dry weight of new terminal shoot growth of Syringa 'Monge' through 28 days

Concentration of ABA (ppm)	Two spray applications, days after removal from storage[a] (gm)			
	2nd & 3rd	6th & 7th	2nd & 3rd 6th & 7th	Means
Control	.48	.51	.50	.50
100	.59	.62	.72	.63
200	.44	.58	.44	.49
Means	.50	.57	.50	.52

Analysis of variance of dry weight

Source of variation	D.F.	S.S.	M.S.	F[b]
Replication	3	.091	.030	.956
Concentration	2	.181	.090	2.822
Time of application	2	.030	.015	.468
Time of application x concentration	4	.062	.015	.487
Error	24	.769	.032	
Total	35	1.13		

[a]Each treatment value is the mean of 12 plants.

[b]F value required at .05 = 3.01.

in dry weight, while two spray application periods at 200 ppm caused a decrease in dry weight. The most effective time of application for increasing dry weight was a 2nd and 3rd and 6th and 7th spray treatment at 100 ppm, while the least effective time of application was a 2nd and 3rd and 6th and 7th spray application at 200 ppm.

Effect of abscisic acid on fresh and dry weight of lateral shoot growth

Fresh and dry weight of lateral shoot growth appears in Tables 11 and 12. Table 11 shows that neither time of application nor concentration was significant. Applications of abscisic acid on the 6th and 7th days at 200 ppm appeared to be the best treatment for increasing fresh weight while the 2nd and 3rd and 6th and 7th spray applications at 100 ppm appeared to be the least effective. The data also show an increase in dry weight as concentration increased.

It can be concluded from the experiment on Syringa 'Monge' that bud break, terminal or lateral, and shoot length, terminal or lateral, are not significantly affected by abscisic acid. It does appear, however, that abscisic acid does cause a slight increase in dry weight of lateral shoot growth as the concentration of abscisic acid is increased.

Table 11. Effect of abscisic acid on fresh weight of new lateral shoot growth of Syringa 'Monge' through 28 days

Concentration of ABA (ppm)	Two spray applications, days after removal from storage[a] (gm)			
	2nd & 3rd	6th & 7th	2nd & 3rd 6th & 7th	Means
Control	.77	.88	.79	.81
100	.91	.99	.57	.83
200	.90	1.10	.84	.94
Means	.86	1.00	.73	.86

Analysis of variance of fresh weight

Source of variation	D.F.	S.S.	M.S.	F[b]
Replication	3	.434	.144	2.14
Concentration	2	.313	.156	2.32
Time of application	2	.476	.238	2.92
Time of application x concentration	4	.162	.040	.60
Error	24	1.61	.067	
Total	35	3.00		

[a] Each treatment value is the mean of 12 plants.

[b] F value required at .05 = 3.01.

Table 12. Effect of abscisic acid on dry weight of new lateral shoot growth of Syringa 'Monge' through 28 days

Concentration of ABA (ppm)	Two spray applications, days after removal from storage[a] (gm)			
	2nd & 3rd	6th & 7th	2nd & 3rd 6th & 7th	Means
Control	.28	.30	.27	.28
100	.29	.35	.19	.28
200	.38	.39	.30	.34
Means	.32	.35	.22	.30

Analysis of variance of dry weight

Source of variation	D.F.	S.S.	M.S.	F[b]
Replication	3	.078	.026	2.04
Concentration	2	.062	.031	2.64
Time of application	2	.041	.020	2.41
Time of application x concentration	4	.019	.004	.56
Error	24	.206	.008	
Total	35	.408		

[a] Each treatment value is the mean of 12 plants.

[b] F value required at .05 = 3.01.

Effect of Abscisic Acid as an Immersion Application on Rosa

Effect of abscisic acid on bud break

'Helen Traubel' roses were immersed in abscisic acid at two concentrations and at four application dates to determine its effect on bud break, shoot elongation, and fresh and dry weight of new shoots.

The effect of abscisic acid on bud break on Rosa 'Helen Traubel' is shown in Table 13. Both concentration and time of application were statistically significant. The concentration of abscisic acid was significant at the .01 level while the time of application was significant at the .05 level. With reference to concentration, an increase in the concentration of abscisic acid resulted in an increase in inhibition of bud break. The results also showed that an application of abscisic acid applied at the time of removal of storage was the most effective in delaying bud break, while applying abscisic acid 4 weeks before removal from storage was the least effective.

Effect of abscisic acid on shoot growth

The effect of abscisic acid on shoot length is shown in Table 14. The data show that the concentration of abscisic acid was statistically significant and, as the concentration increased, there was a decrease in shoot length. The data also show that both concentrations of abscisic acid were effective in inhibiting shoot length as compared to the control. Time of application showed no effect on shoot growth.

Table 13. Effect of abscisic acid on days to bud break of Rosa 'Helen Traubel' through 28 days

Concentration of ABA (ppm)	Immersion applications, weeks before removal from storage[a] (days)				
	4 weeks	4 & 0 weeks	3 & 2 weeks	0 weeks[b]	Means
Control	9.0	8.8	10.3	12.4	10.1
200	14.5	15.3	13.8	13.9	14.3[c]
400	12.7	15.1	14.1	16.5	14.6[c]
Means	12.0	13.0	12.5	14.3[d]	13.0

Analysis of variance of bud break

Source of variation	D.F.	S.S.	M.S.	F
Replication	3	15.27	5.09	1.40
Concentration	2	198.60	99.30	27.34**
Time of application	3	31.53	10.51	2.89*
Time of application x concentration	6	39.51	6.58	1.81
Error	33	119.85	3.63	
Total	47	404.79		

**Significant at .01 level.

*Significant at .05 level.

[a]Each treatment value is the mean of 16 plants.

[b]Applied at time of removal from storage.

[c]LSD (.01) = 1.9.

[d]LSD (.05) = 1.3.

Table 14. Effect of abscisic acid on length of new shoot growth of Rosa 'Helen Traubel' through 28 days

Concentration of ABA (ppm)	Immersion applications, weeks before removal from storage[a] (cm)				
	4 weeks	4 & 0 weeks	3 & 2 weeks	0 weeks	Means
Control	24.5	27.6	25.2	24.1	25.3
200	19.1	18.4	19.2	16.6	18.3[b]
400	19.6	19.9	17.7	16.1	18.4[b]
Means	21.1	22.0	21.0	19.0	20.8

Analysis of variance of shoot growth

Source of variation	D.F.	S.S.	M.S.	F
Replication	3	119.42	39.83	1.99
Concentration	2	521.22	260.61	13.05**
Time of application	3	57.31	19.10	.95
Time of application x concentration	6	25.11	4.18	.20
Error	33	658.84	19.96	
Total	47	1381.99		

**Significant at .01 level.

[a]Each treatment value is the mean of 16 plants.

[b]LSD (.01) = 4.3.

Effect of abscisic acid on fresh and dry weight

Data in Tables 15 and 16 show that the concentration of abscisic acid was significant at the .01 level in influencing both fresh and dry weight. Data on fresh weight also indicate that as the concentration of abscisic acid increased, there was a decrease in fresh weight regardless of time of application. The results also show that an application 0 weeks before removal from storage was the most effective.

The results on dry weight of shoot growth show a decreasing response as the concentration of abscisic acid was increased, but this trend does not occur with respect to the time of application.

It can be concluded from this data that time of application and concentration does affect bud break. The results also indicate that concentration of abscisic acid does affect shoot growth, but time of application resulted in no significant difference. Finally, abscisic acid does affect fresh and dry weight of shoot growth, but time of application was not a factor.

Effect of Abscisic Acid as an Immersion Application on Syringa

Effect of abscisic acid on terminal bud break

The effect of abscisic acid on terminal bud break is shown in Table 17. Concentration was statistically significant at the .01 level, while time of application was not significant. Table 17 also shows that as concentration increased, there was

Table 15. Effect of abscisic acid on fresh weight of new shoot growth of Rosa 'Helen Traubel' through 28 days

Concentration of ABA (ppm)	Immersion applications, weeks before removal from storage[a] (gm)				
	4 weeks	4 & 0 weeks	3 & 2 weeks	0 weeks	Means
Control	7.0	7.6	7.2	6.4	7.1
200	5.3	5.2	5.8	4.5	5.2[b]
400	5.5	5.5	5.1	4.4	5.1[b]
Means	6.0	6.1	6.0	5.1	5.8

Analysis of variance of fresh weight

Source of variation	D.F.	S.S.	M.S.	F
Replication	3	18.60	6.20	2.76
Concentration	2	37.93	18.96	8.46**
Time of application	3	7.48	2.49	1.11
Time of application x concentration	6	2.07	.34	.15
Error	33	73.96	2.24	
Total	47	140.07		

**Significant at .01 level.

[a]Each treatment value is the mean of 16 plants.

[b]LSD (.01) = 1.4.

Table 16. Effect of abscisic acid on dry weight of new shoot growth of Rosa 'Helen Traubel' through 28 days

Concentration of ABA (ppm)	Immersion applications, weeks before removal from storage[a] (gm)				
	4 weeks	4 & 0 weeks	3 & 2 weeks	0 weeks	Means
Control	1.3	1.6	1.3	1.20	1.40
200	.9	.8	1.0	.79	.90[b]
400	.9	1.0	.9	.79	.93[b]
Means	1.1	1.2	1.1	.94	1.00

Analysis of variance of dry weight

Source of variation	D.F.	S.S.	M.S.	F
Replication	3	1.14	.38	4.11*
Concentration	2	2.39	1.19	12.85**
Time of application	3	.31	.10	1.14
Time of application x concentration	6	.21	.03	.38
Error	33	3.06	.09	
Total	47	7.13		

**Significant at .01 level.

*Significant at .05 level.

[a]Each treatment value is the mean of 16 plants.

[b]LSD (.01) = .27.

Table 17. Effect of abscisic acid on days to terminal bud break of Syringa 'Monge' through 28 days

Concentration of ABA (ppm)	Immersion applications, weeks before removal from storage[a] (days)				
	4 weeks	4 & 0 weeks	3 & 2 weeks	0 weeks[b]	Means
Control	5.1	6.8	6.6	7.0	6.4
200	9.2	11.0	11.2	7.4	9.7[c]
400	11.9	11.5	11.2	7.5	10.5[c]
Means	8.7	9.8	9.7	7.3	8.8

Analysis of variance of terminal bud break

Source of variation	D.F.	S.S.	M.S.	F
Replication	3	64.55	21.51	1.58
Concentration	2	154.29	77.14	5.70**
Time of application	3	47.38	15.79	1.16
Time of application x concentration	6	51.42	8.57	0.63
Error	33	446.62	13.53	
Total	47	764.28		

**Significant at .01 level.

[a]Each treatment value is the mean of 12 plants.

[b]Applied at time of removal from storage.

[c]LSD (.05) = 3.1.

a delay in days to bud break, regardless of time of application. Although time of application was not significant, two applications of abscisic acid, rather than one, were more beneficial in preventing terminal bud break.

Effect of abscisic acid on lateral bud break

Data on lateral bud break are shown in Table 18. The results show that there is an increase in inhibition as the concentration of abscisic acid was increased, while time of application showed no noticeable response. Replication, concentration, and the time of application x concentration were significant at the .01 level, while time of application was significant at the .05 level. The results indicate that the use of two applications of abscisic acid was more effective than one application in delaying bud break.

Effect of abscisic acid on terminal and lateral shoot growth

Terminal and lateral shoot growth in Tables 19 and 20 show no statistical significance in either time of application or concentration. Although concentration was not significant, terminal shoot growth showed a decreasing response with regard to time of application. Table 19 also shows that applying abscisic acid at the end of storage caused greater reduction in shoot growth than if applied at the beginning of storage.

Data on lateral shoot growth indicated that neither time of application nor concentration of abscisic acid was

Table 18. Effect of abscisic acid on days to lateral bud break of Syringa 'Monge' through 28 days

Concentration of ABA (ppm)	Immersion applications, weeks before removal from storage[a] (days)				
	4 weeks	4 & 0 weeks	3 & 2 weeks	0 weeks	Means
Control	11.7	11.1	15.7	8.2	11.7
200	12.2	17.7	13.6	16.3	14.9
400	16.8	16.8	15.4	10.6	14.9
Means	13.6	15.2	14.9	11.7	13.8

Analysis of variance of lateral bud break

Source of variation	D.F.	S.S.	M.S.	F
Replication	3	131.14	43.71	4.33**
Concentration	2	110.55	55.27	5.47**
Time of application	3	90.67	30.22	2.99*
Time of application x concentration	6	201.80	33.63	3.33*
Error	33	332.88	10.08	
Total	47	867.07		

**Significant at .01 level.

*Significant at .05 level.

[a]Each treatment value is the mean of 12 plants.

Table 19. Effect of abscisic acid on length of new terminal shoot growth of Syringa 'Monge' through 28 days

Concentration of ABA (ppm)	Immersion applications, weeks before removal from storage[a] (cm)				
	4 weeks	4 & 0 weeks	3 & 2 weeks	0 weeks	Means
Control	10.0	7.3	5.9	6.4	7.4
200	6.6	7.1	8.4	7.0	7.3
400	9.3	8.5	7.5	6.4	7.9
Means	8.6	7.6	7.3	6.6	7.5

Analysis of variance of terminal length

Source of variation	D. F.	S.S.	M.S.	F[b]
Replication	3	23.77	7.92	1.39
Concentration	2	3.78	1.89	0.33
Time of application	3	24.89	8.29	1.46
Time of application x concentration	6	39.45	6.57	1.15
Error	33	187.47	5.68	
Total	47	279.38		

[a]Each treatment value is the mean of 12 plants.

[b]F value required at .05 = 2.98.

Table 20. Effect of abscisic acid on length of new lateral shoot growth of Syringa 'Monge' through 28 days

Concentration of ABA (ppm)	Immersion applications, weeks before removal from storage[a] (cm)				
	4 weeks	4 & 0 weeks	3 & 2 weeks	0 weeks	Means
Control	7.0	5.8	5.9	5.8	6.1
200	5.9	4.9	6.1	6.6	5.9
400	7.9	5.1	6.9	5.7	6.4
Means	6.9	5.3	6.3	6.0	6.1

Analysis of variance in lateral length

Source of variation	D.F.	S.S.	M.S.	F[b]
Replication	3	13.48	4.49	1.13
Concentration	2	2.27	1.13	0.28
Time of application	3	16.41	5.47	1.38
Time of application x concentration	6	11.74	1.95	0.49
Error	33	130.37	3.95	
Total	47	174.28		

[a]Each treatment value is the mean of 12 plants.

[b]F value required at .05 = 2.98.

statistically significant. The results also showed that 400 ppm of abscisic acid appeared to increase lateral shoot growth, while 200 ppm of abscisic acid caused a slight reduction in shoot growth as compared to the control.

Effect of abscisic acid on fresh and dry weight of terminal shoot growth

Fresh and dry weight of terminal shoot growth, Tables 21 and 22, show no significant difference with regard to time of application or concentration. However, a concentration of 400 ppm abscisic acid caused an increase in fresh weight, while 200 ppm caused a slight decrease in fresh weight as compared to the control. The data also indicate that an application of abscisic acid four weeks before removal from storage was the most effective treatment in increasing fresh weight.

Table 22, on dry weight, shows a pattern similar to fresh weight. An application of abscisic acid four weeks before removal from storage at 400 ppm was the most effective treatment in increasing dry weight.

Effect of abscisic acid on fresh and dry weight of lateral shoot growth

Data on fresh weight and dry weight of lateral shoot growth appear in Tables 23 and 24. With reference to statistical significance, neither time of application nor concentration caused a significant change in fresh weight or dry weight of lateral shoot growth.

Table 21. Effect of abscisic acid on fresh weight of new terminal shoot growth of Syringa 'Monge' through 28 days

Concentration of ABA (ppm)	Immersion applications, weeks before removal from storage[a] (gm)				
	4 weeks	4 & 0 weeks	3 & 2 weeks	0 weeks	Means
Control	1.6	1.3	1.4	1.3	1.4
200	1.2	1.1	1.6	1.2	1.3
400	2.0	1.7	1.5	1.1	1.6
Means	1.6	1.4	1.5	1.2	1.4

Analysis of variance of terminal fresh weight

Source of variation	D.F.	S.S.	M.S.	F[b]
Replication	3	2.04	0.68	2.06
Concentration	2	0.76	0.38	1.14
Time of application	3	1.10	0.36	1.11
Time of application x concentration	6	1.34	0.22	0.67
Error	33	10.92	0.33	
Total	47	16.18		

[a]Each treatment value is the mean of 12 plants.

[b]F value required at .05 = 2.98.

Table 22. Effect of abscisic acid on dry weight of new terminal shoot growth of Syringa 'Monge' through 28 days

Concentration of ABA (ppm)	Immersion applications, weeks before removal from storage[a] (gm)				
	4 weeks	4 & 0 weeks	3 & 2 weeks	0 weeks	Means
Control	0.57	0.45	0.47	0.39	0.47
200	0.40	0.46	0.55	0.40	0.45
400	0.67	0.52	0.49	0.37	0.51
Means	0.55	0.48	0.50	0.39	0.47

Analysis of variance of fresh weight

Source of variation	D.F.	S.S.	M.S.	F[b]
Replication	3	.033	.011	0.33
Concentration	2	.031	.015	0.45
Time of application	3	.164	.054	1.61
Time of application x concentration	6	.145	.024	0.71
Error	33	1.12	.033	
Total	47	1.49		

[a]Each treatment value is the mean of 12 plants.

[b]F value required at .05 = 2.98.

Table 23. Effect of abscisic acid on fresh weight of new lateral shoot growth of Syringa 'Monge' through 28 days

Concentration of ABA (ppm)	Immersion applications, weeks before removal from storage[a] (gm)				
	4 weeks	4 & 0 weeks	3 & 2 weeks	0 weeks	Means
Control	1.0	.87	.9	.91	.94
200	.9	.84	.9	.98	.92
400	1.5	.80	1.2	.94	1.10
Means	1.2	.84	1.0	.95	.95

Analysis of variance of fresh weight

Source of variation	D.F.	S.S.	M.S.	F[b]
Replication	3	.845	.28	1.28
Concentration	2	.391	.19	.89
Time of application	3	.644	.21	.98
Time of application x concentration	6	.671	.11	.51
Error	33	7.22	.21	
Total	47	9.77		

[a]Each treatment value is the mean of 12 plants.

[b]F value required at .05 = 2.98.

Table 24. The effect of abscisic acid on dry weight of new lateral shoot growth of Syringa 'Monge' through 28 days

Concentration of ABA (ppm)	Immersion applications, weeks before removal from storage[a] (gm)				
	4 weeks	4 & 0 weeks	3 & 2 weeks	0 weeks	Means
Control	.26	.30	.34	.31	.30
200	.31	.25	.29	.32	.29
400	.51	.26	1.0	.31	.53
Means	.36	.27	.55	.31	.37

Analysis of variance of dry weight

Source of variation	D.F.	S.S.	M.S.	F[b]
Replication	3	.41	.138	.88
Concentration	2	.56	.284	1.81
Time of application	3	.56	.187	1.19
Time of application x concentration	6	.95	.159	1.01
Error	33	5.17	.156	
Total	47	7.68		

[a]Each treatment value is the mean of 12 plants.

[b]F value required at .05 = 2.98.

Data on fresh weight show that 400 ppm of abscisic acid resulted in an increase of fresh weight while 200 ppm caused a slight decrease in fresh weight. Although not significant, an application of abscisic acid 4 weeks before removal from storage was the most effective treatment in increasing fresh weight (Table 23).

Although not significant, Table 24 shows that 400 ppm of abscisic acid tended to cause an increase in dry weight. The results also indicate that the best treatment in increasing dry weight was an application of abscisic acid at 3 and 2 weeks before removal from storage.

In conclusion, the results of the experiment indicate that abscisic acid does delay terminal and lateral bud break. The experiment also shows that abscisic acid has no significant growth effect on shoot growth, fresh weight, or dry weight of Syringa 'Monge.'

Effect of Abscisic Acid as an Immersion Application on Bud Break of Rosa 'First Prize'

The purpose of this experiment was to determine if varying the time of the immersion application of abscisic acid had any effect in delaying bud break.

Data on bud break appear in Figure 1. There was an increasing response with increasing the time of immersion. The most effective treatment in this experiment appears to be a 9-minute immersion application while a 5-second immersion was least effective. From this experiment, it can be concluded

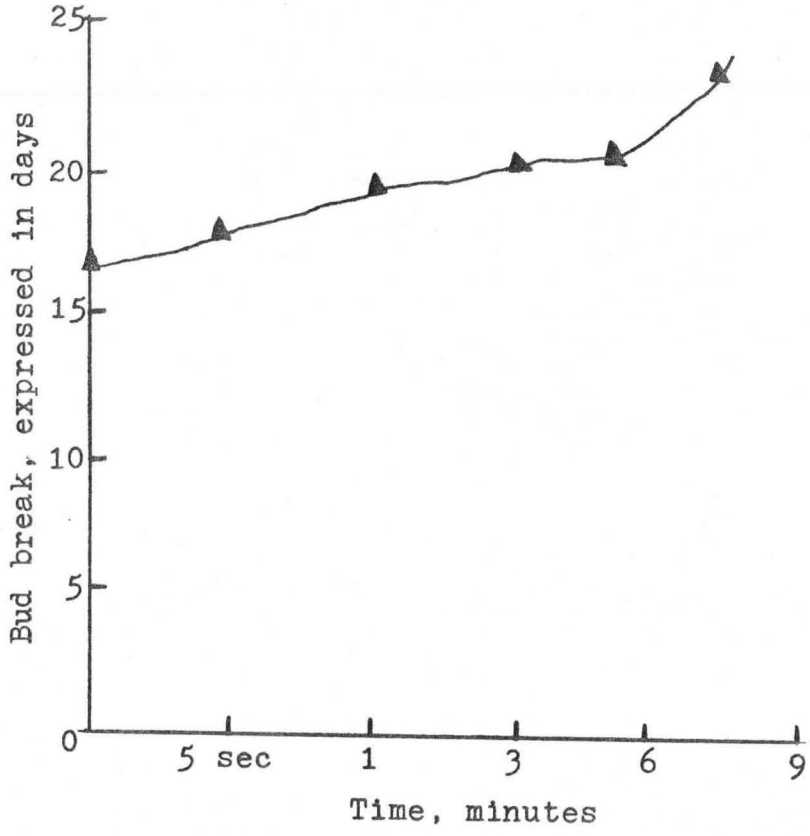

Figure 1. Effect of 400 ppm of abscisic acid on bud break of Rosa 'First Prize'

that the longer a plant is in contact with abscisic acid the greater the delay in bud break.

Effect of Abscisic Acid on *Lactuca sativa* 'Grand Rapids'

The effect of abscisic acid on percent germination and radicle length of 'Grand Rapids' lettuce is shown in Table 25 and Figures 2 and 3. Data in Table 25 and Figure 2 show a decrease in percent germination as the concentration of abscisic acid increased in both solutions 1 and 2. Figure 3 indicates that a decreasing linear response of radicle length occurred as the concentration of abscisic acid increased.

During the second and twelfth weeks of this experiment, it appeared that solution 2 was slightly more effective in decreasing percent germination than solution 1. With regard to radicle length, solution 2 at 12 weeks was slightly more effective in inhibiting radicle length than was solution 1 at lower concentrations; while at higher concentrations (5.0-10.0 ppm), there seemed to be no difference between the solutions. The data also show that there is a slight decrease in biological activity of abscisic acid in both solutions 1 and 2 from the second to the twelfth week.

Table 25. Effect of storage time and concentration on abscisic acid degradation at 37° F as measured in 'Grand Rapids' lettuce seed bioassay

	Lettuce seed bioassay (expressed in cm and %)							
	2 weeks				12 weeks			
	Solution 1[a]		Solution 2[b]		Solution 1[a]		Solution 2[b]	
Concentration of ABA	Percent germination	Radicle length	Percent germination	Radicle length	Percent germination	Radicle length	Percent germination	Radicle length
Control	97.5	4.35	97.5	4.35	100	4.71	100	4.71
.5 ppm	95.0	2.71	90.0	3.16	98	4.12	94	3.57
1.0 ppm	97.5	2.05	80.5	2.37	96	2.90	96	2.05
2.5 ppm	77.5	.50	37.5	.40	68	.60	58	.60
5.0 ppm	0.0	0.00	12.5	.10	10	.10	14	.20
10.0 ppm	0.0	0.00	0.0	0.00	0	0.00	0	0.00

[a]Solution previously not used in other experiments.

[b]Solution previously used in other experiments.

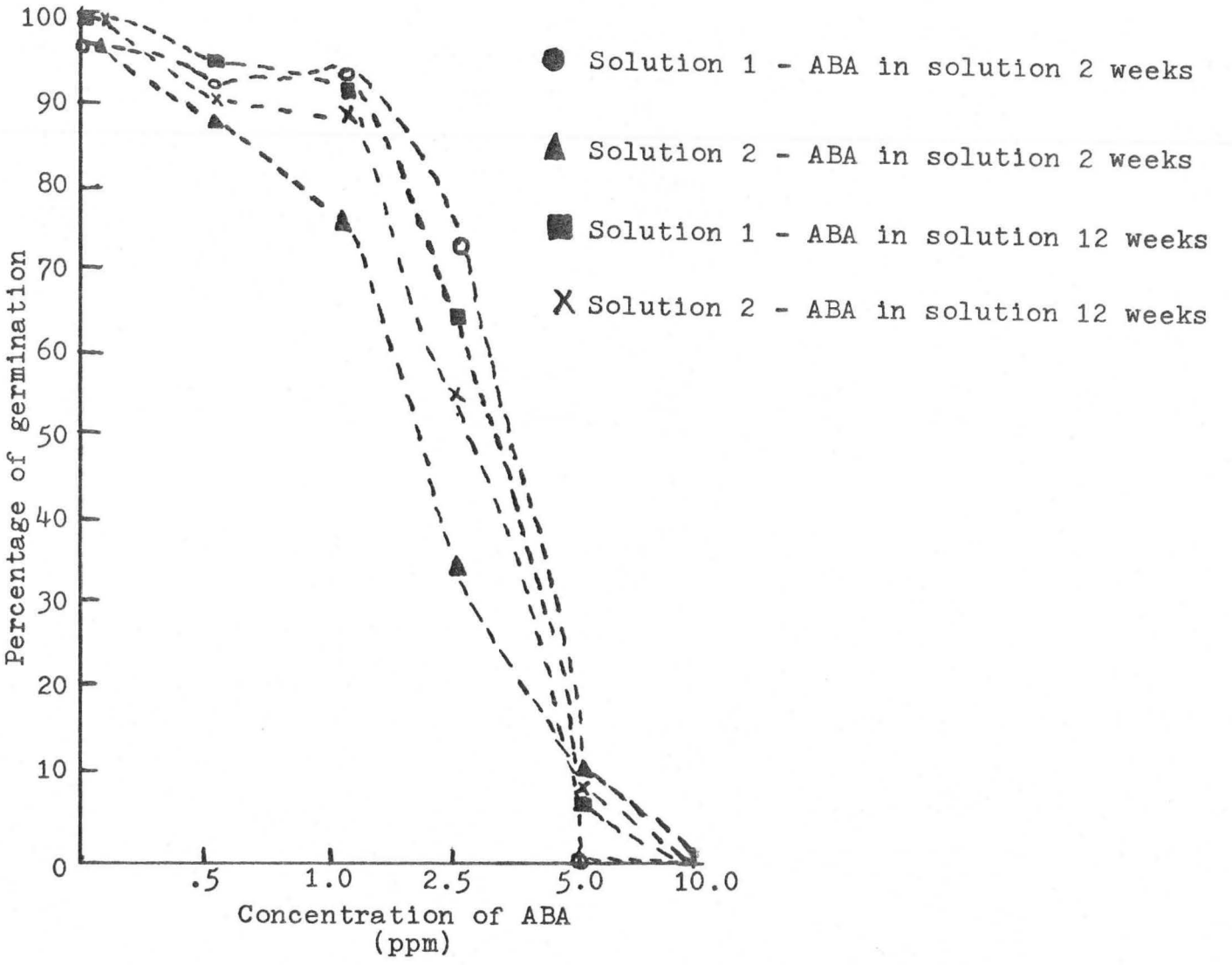

Figure 2. The effect of the biological activity of abscisic acid in solution during storage (37° F) on germination of Lactuca sativa 'Grand Rapid'

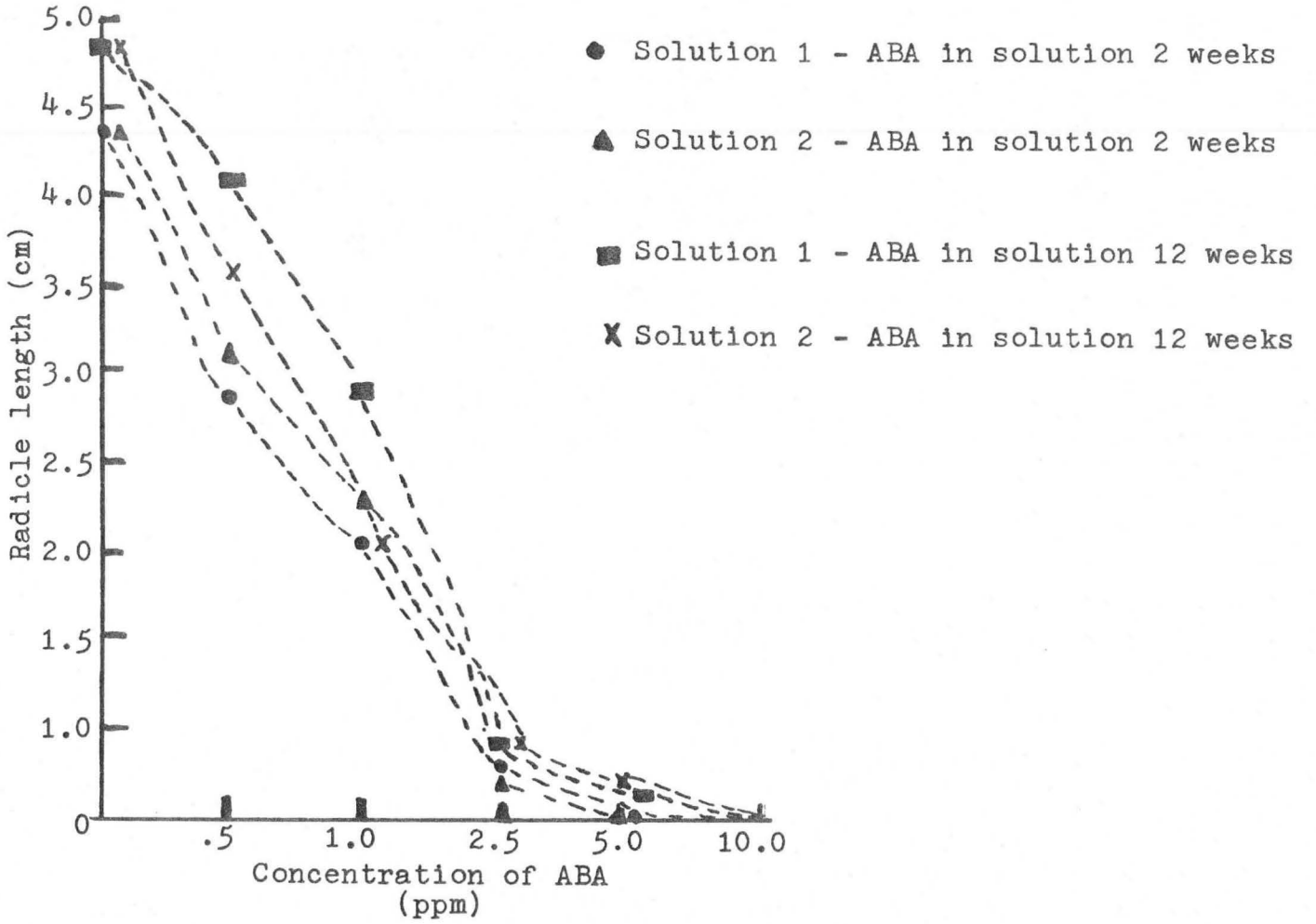

Figure 3. The effect of the biological activity of abscisic acid in solution during storage (37° F) on radicle growth of Lactuca sativa 'Grand Rapid'

DISCUSSION

The effect of abscisic acid at various concentrations, time of application, and methods of application on Rosa 'Helen Traubel' and Syringa 'Monge' indicate that abscisic acid affected bud break, shoot elongation, fresh weight and dry weight of shoot growth. Abscisic acid showed differences in its effectiveness as determined by the method of application, concentration and, in a few cases, by the time of application.

With reference to the spray application of abscisic acid on Syringa 'Monge,' it can be concluded that abscisic acid did not significantly inhibit bud break or alter any observed physiological response. Abscisic acid did significantly delay bud break of Rosa 'Helen Traubel' but did not alter other observed growth responses.

El-Antably et al. (23) reported that when abscisic acid was applied in the form of a spray at 5 and 25 ppm to dormant peach trees, it did not delay flower bud break. Apple and pear buds were also treated with abscisic acid at 5 and 20 ppm during April and May, but such treatment caused no significant delay in bud break. Abscisic acid at 5 or 25 ppm was applied to cut shoots of apple, black currants, poplar, grape, birch, plum, and pear for 15 days. In all cases, abscisic acid did not delay bud break. Meyer[1] has reported that abscisic acid

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[1]Meyer, M. M., University of Illinois. Information on prolonging dormancy with abscisic acid. October, 1969.

in the form of a spray did not prolong dormancy in common storage or increase growth after storage in dormant plants of Forsythia intermedia, Rosa hybrida, Rosa multiflora, and Cornus alba siberica.

A possible speculation why abscisic acid did not alter most of the observed growth responses may have been caused by two major factors. First, it is possible that an insufficient amount of abscisic acid penetrated the buds and stems which would have resulted in a lack of response. El-Antably et al. (23) reported that poor penetration of abscisic acid into the wood of Ribes nigrum and Acer pseudoplatanus prevented prolonging dormancy, but with repeated spray applications of abscisic acid, they were able to extend dormancy. Little and Edict (42) have reported that abscisic acid did not delay bud break when applied as a spray application, but did delay bud break when applied as a soil drench to potted fir trees.

A second reason for a lack of response in sprayed plants as compared to the immersed plants may have been due to a seasonal interaction with gibberellic acid. Kawase (38) and Blommaert (6) have shown that from seasonal extracts of buds, especially of those in the spring, abscisic acid is at a very low level in the bud while gibberellic acid is at a much higher level. If this is the case, one might assume that in Rosa a small amount of abscisic acid entered the buds and caused the inhibition of gibberellic acid biosynthesis. Secondly, abscisic acid could have delayed bud break by inhibition of

either DNA or RNA which in turn inhibited the biosynthesis of gibberellic acid. Work of Wareing *et al.* (64) on spinach and corn supports a theory that inhibition of growth is caused by a reduction of gibberellic biosynthesis while the work of Overbeek *et al.* (52) on *Lemna minor* has shown that abscisic acid prevented the synthesis of DNA.

Others such as Villiers (63) and Staden and Bornman (56) believe this reduction of growth is due to an inhibition of RNA. With the hypothesis that one of the above cases is true, a small quantity of abscisic acid entered the buds and stems of *Rosa* causing an inhibition of growth for a short duration and later was either broken down or counteracted by gibberellic acid or some other specific hormone which later resulted in resumption of DNA or RNA synthesis. Staden and Bornman (56) have reported that abscisic acid inhibits growth of *Spirodela*. Resumption of growth in *Spirodela* is believed to be caused by an inactivation of abscisic acid resulting in the lowering of its concentration and resumption of nucleic acid metabolism.

The effect of abscisic acid on *Syringa* 'Monge' in the form of an immersion application indicates that abscisic acid was effective in inhibition of terminal and lateral bud break, but that it had no significant effect on growth after bud break. Results also indicated, as in the case of spray treatment of *Rosa* 'Helen Traubel,' that abscisic acid caused a short duration of inhibition of growth. Upon the counteracting of

abscisic acid by some growth promoter, further inhibition ceased and growth commenced.

With regard to the immersion application on Rosa 'Helen Traubel,' abscisic acid did affect bud break, shoot elongation, and fresh and dry weight of shoot elongation. In all cases, concentration was the major influence in inhibiting these physiological responses rather than the time of application.

Little and Edict (42) reported that abscisic acid at 10 ppm inhibited bud break in ash and maple cuttings. At .4 and 2.0 ppm, it had no effect on time to bud break. Young and Cooper (67) reported that abscisic acid delayed bud break of Red Blush grapefruit seedlings at 100 ppm, and higher concentrations further increased the delay. They also showed that the number of applications at a given concentration also influenced bud break. At even higher concentrations, it caused injury to wood and in some cases killed the plants.

It is also concluded that a greater penetration of abscisic acid occurred in the immersion application than in the spray application of Rosa since both methods of application at 200 ppm were effective in inhibiting bud break, but only the immersion application was effective in inhibiting shoot elongation and fresh and dry weight of shoot growth. It can be interpreted from these results that the most effective penetration of abscisic acid into the buds and stems did result in inhibition of subsequent growth of the plants.

Since a 5-minute immersion application was superior to the spray application, the effect of abscisic acid on Rosa 'First Prize' was used to determine if time of immersion had any influence in affecting bud break. From Figures 2 and 3 it appears that increasing the time of application did result in delaying bud dormancy. A 9-minute immersion of abscisic acid was the most effective treatment, while a 5-second immersion was the least effective. It can be concluded that greater penetration of abscisic acid at a given level does occur as the time in which the plant is in contact with abscisic acid is increased.

The effect of abscisic acid on lettuce seed germination was used to determine if a decrease in biological activity of abscisic acid occurred during storage. The results indicated that there was only a slight decrease in biological activity of abscisic acid after 12 weeks in storage (37° F). Decrease in biological activity of abscisic acid over this period may have been due either to the continuous exposure to light or possibly due to exposing the solutions to room temperature for long periods of time. It has been reported by Milborrow[1] that abscisic acid must be kept at 37° F in methanol and in darkness once in solution or the biological activity will be reduced.

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[1]Milborrow, B. V., Kent, England. Information on biological activity of abscisic acid in storage. Private communication. December, 1969.

SUMMARY

The influence of abscisic acid in controlling bud break, shoot elongation, fresh weight, and dry weight of shoot growth was investigated in both Rosa 'Helen Traubel' and Syringa 'Monge.' The effect of duration of an application of abscisic acid as well as the biological activity of abscisic acid during storage were also determined.

Abscisic acid, when applied in the form of a spray application, was found to inhibit bud break of Rosa 'Helen Traubel' but did not significantly affect bud break of Syringa 'Monge.' It was also determined that abscisic acid did not significantly affect shoot elongation, fresh weight, or dry weight of shoot growth in either Rosa or Syringa. However, it did appear that in most cases, as the concentration of abscisic acid was increased, there was a greater inhibition of growth. It was also concluded that the time of application did not significantly influence Rosa or Syringa.

Abscisic acid, when applied in the form of an immersion application, was found to significantly delay bud break in Rosa 'Helen Traubel' and Syringa 'Monge.' In all cases, 400 ppm of abscisic acid caused the greatest inhibition of bud break. Shoot elongation, fresh weight, and dry weight of Rosa were found to be significantly inhibited, but abscisic acid did not significantly influence shoot elongation, fresh weight, or dry weight of shoot growth of Syringa. Time of application

in all but one case was found not to affect any of these observed physiological responses.

Abscisic acid, when applied to Rosa 'First Prize' at various durations, was found to affect bud break. It appeared that the longer the roses were in contact with abscisic acid, the greater the inhibition of bud break.

Lettuce seeds were used to indicate the biological activity of abscisic acid as a means of percent germination and radicle length. The biological activity of abscisic acid in a lettuce seed bioassay was shown to have decreased slightly after 12 weeks in storage at 37° F.

It is proposed from this study that abscisic acid does influence certain physiological processes of Rosa and Syringa. It is also concluded that the concentration of abscisic acid rather than time of application is the major determinant in affecting these observed physiological responses.

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